

AR201-14112A

Group 16 - Alkenyl Succinic Anhydride Category
November 22, 2002

HIGH PRODUCTION VOLUME (HPV)

CHALLENGE PROGRAM

TEST PLAN

For

ALKENYL SUCCINIC ANHYDRIDE CATEGORY

**Prepared by
The American Chemistry Council
Petroleum Additives Panel
Health, Environmental, and Regulatory Task Group**

2002 DEC 10 PM 12:21
RECEIVED
OFFICE

November 22, 2002

**LIST OF MEMBER COMPANIES IN THE
HEALTH, ENVIRONMENTAL, AND REGULATORY TASK GROUP**

The Health, Environmental, and Regulatory Task Group (HERTG) of the American Chemistry Council Petroleum Additives Panel includes the following member companies:

B.P. PLC

Chevron Oronite Company, LLC

Crompton Corporation

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

Rhodia, Inc.

EXECUTIVE SUMMARY

The American Chemistry Council Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its member companies, hereby submit for review and public comment their test plan for the "*Alkenyl Succinic Anhydride*" category of chemicals under the United States Environmental Protection Agency High Production Volume (HPV) Chemical Challenge Program. This report should be read in its entirety in order to obtain an understanding of the chemical category and proposed testing.

Alkenyl Succinic Anhydride Category. Relying on several factors specified in the EPA guidance document on "Development of Chemical Categories in the HPV Challenge Program," in which use of chemical categories is encouraged, the following three closely related chemicals constitute a chemical category as used by the petroleum additives industry:

- 2,5-Furandione, dihydro-3-(tetrapropenyl)-, (CAS #26544-38-7), referred to as "tetrapropenylsuccinic anhydride".
- Butanedioic acid, (tetrapropenyl)-, (CAS #27859-58-1), referred to as "tetrapropenyl butanedioic acid".
- 2,5-Furandione, 3-(dodecenyl) dihydro-, (CAS #25377-73-5), referred to as "dodecenylsuccinic anhydride".

Although the United States Toxic Substances Control Act - Inventory Update Rule (IUR) report lists 1990 production volumes for *straight-chain* C12 (i.e., dodecyl) succinic anhydride, additive manufacturers synthesize *branched* C12 (i.e., tetrapropenyl) succinic anhydride using propene tetramer. Although several studies identify the dodecyl derivative as the test substance, the tetrapropenyl derivative is and always has been the manufactured chemical. In this test plan, CAS #25377-73-5 and CAS #26544-38-7 are considered interchangeable and refer to the same substance, tetrapropenylsuccinic anhydride. It is also important to emphasize that tetrapropenylsuccinic anhydride (CAS #26544-38-7) can hydrolyze to tetrapropenyl butanedioic acid (CAS #27859-58-1). Because under many conditions the diacid form of the anhydride will be the form that exists and drives concern, consideration of both is necessary to adequately assess hazard for this category. For this reason, the anhydride and diacid are included together in this test plan, although they are chemically dissimilar.

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity. Substances in this category consist of either a succinic anhydride structure or a butanedioic acid with a tetrapropenyl substituent group. The anhydride can undergo hydrolysis to the butanedioic acid.

Similarity of Physicochemical Properties. The relative similarity of selected physicochemical properties of these substances is consistent with their generally similar structure. These substances have low volatility due to their low vapor pressure (typically $\leq 3\text{E-}4$ Pa @25°C), high viscosity (typically 200 cSt @ 25°C), densities of approximately 1.0 g/ml (@25°C), sparingly low water solubility (<10 mg/L based on calculated data), and molecular weights of 266.4 or 284.4 daltons.

Fate and Transport Characteristics. A member of this category, tetrapropenyl butanedioic acid, has been shown to have limited biodegradability. Existing information for the anhydride suggests it will undergo hydrolysis and will be the form that should be considered when evaluating environmental fate. Direct photodegradation is not expected to cause significant physical degradation of members in this category. However, computer-modeled data will be developed to adequately characterize their potential to oxidize as a result of hydroxyl (OH-) radical attack. These substances are not expected to significantly partition to air if released into the environment because of their relatively low vapor pressure. Computer-modeled environmental partitioning data will be calculated for members of this category to determine their potential to partition to other environmental compartments.

Toxicological Similarity. Review of reliable published and unpublished test data for members of the alkenyl succinic anhydride category suggests that the toxicity profiles of these chemicals are similar. Data obtained from proposed additional testing will further characterize the toxicological endpoints in the HPV Challenge Program for all members within this category.

Aquatic Toxicology. Alga toxicity data for a member of the category were reviewed, and the findings indicate some toxicity when appropriate test methods are used. Additional testing is proposed to more fully characterize the aquatic toxicity potential for members of the category.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity were reviewed, and the findings indicate a low concern for acute toxicity. Data are available for two members of the category indicating that the category has been well tested for acute mammalian effects. Therefore, no additional acute mammalian toxicity testing is necessary.

Mammalian Toxicology - Mutagenicity. Valid data from bacterial reverse mutation assays and *in vitro* chromosome aberration studies were not located.

Mammalian Toxicology - Subchronic Toxicity. Valid data from repeated-dose toxicity studies were not located.

Mammalian Toxicology - Reproductive and Developmental Toxicity. Valid data from a reproductive/developmental toxicity screening study were not located.

Conclusion. Based upon the data reviewed for this test plan, the physicochemical, environmental fate, and toxicological properties of category members are similar and/or follow a predictable pattern based on structure. This category contains an anhydride and its hydrolytic reaction product, which upon first review may be questionable to include in a single category, but it is the opinion of the authors of this test plan that these two chemicals cannot be considered separately with regard to toxicity and environmental fate. Therefore, the EPA definition of a chemical category has been met, and the three CAS numbers that constitute the alkenyl succinic anhydride category will be evaluated in accordance with the test plan summarized below.

Test Plan. The test plan for the alkenyl succinic anhydride category includes the following testing, computer modeling, or technical discussions:

- Physicochemical - The water solubility of tetrapropenyl butanedioic acid (CAS #27859-58-1) will be determined.
- Hydrolysis - The potential for tetrapropenylsuccinic anhydride (CAS #26544-38-7) to hydrolyze will be characterized. The public and available private literature will be evaluated to determine whether there is sufficient information to adequately characterize the potential hydrolysis rate of tetrapropenylsuccinic anhydride (CAS #26544-38-7). If it is determined that there is a lack of adequate information, this substance will be tested to develop hydrolytic rate data. If sufficient information is available in the general literature, it will be provided in the form of a robust summary.
- Photodegradation - The chemical structure of category members will be evaluated to determine whether there is a potential for direct photodegradation. Data will also be developed to characterize indirect photodegradation for category members using the AOP model in EPIWIN. Information or data for both routes of degradation will be provided in robust summaries.
- Fugacity modeling - Environmental partitioning data for members of this category will be calculated using a Mackay Level I equilibrium partitioning model and provided in robust summaries.
- Acute fish toxicity - Testing will be conducted on tetrapropenyl butanedioic acid (CAS #27859-58-1). Results will be bridged to other members of the category.
- Acute invertebrate toxicity - Testing will be conducted on tetrapropenyl butanedioic acid (CAS #27859-58-1). Results will be bridged to other members of the category.
- Mutagenicity - Bacterial mutation and *in vitro* chromosome aberration studies will be conducted on the tetrapropenyl butanedioic acid derivative (CAS #27859-58-1). Results will be bridged to other members of the category.
- Repeated-dose toxicity - Tetrapropenyl butanedioic acid (CAS #27859-58-1) will be tested in a 28-day dose-range finding study for the reproductive/developmental toxicity study.
- Reproductive/developmental toxicity - Tetrapropenyl butanedioic acid (CAS #27859-58-1) will be tested in a one-generation study.

As this test plan was developed, careful consideration was given to the number of animals that would be required for tests included in the proposed plan and conditions to which the animals might be exposed. In consideration of the concerns of some non-governmental organizations about animal welfare, the use of animals in this proposed test plan has been minimized.

TABLE OF CONTENTS

1.0	INTRODUCTION	1
2.0	CHEMISTRY OF ALKENYL SUCCINIC ANHYDRIDES	3
2.1	DESCRIPTION	3
2.2	PHYSICOCHEMICAL PROPERTIES	4
2.2.1	Molecular Weight	4
2.2.2	Specific Gravity	4
2.2.3	Melting Point and Boiling Point	4
2.2.4	Vapor Pressure and Viscosity	4
2.2.5	Water Solubility	5
2.2.6	Octanol-Water Partition Coefficient	5
3.0	USES OF THE ALKENYL SUCCINIC ANHYDRIDE CATEGORY.....	5
4.0	EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA.....	6
4.1	ENVIRONMENTAL FATE DATA	6
4.1.1	Physicochemical Properties Relevant to Environmental Fate	6
4.1.2	Biodegradation.....	7
4.1.2.1	Test Methodologies	7
4.1.2.2	Summary of Available Data	7
4.1.2.3	Data Assessment and Test Plan for Biodegradability	7
4.1.3	Hydrolysis.....	8
4.1.3.1	Test Methodologies	8
4.1.3.2	Summary of Available Data	8
4.1.3.3	Data Assessment and Test Plan for Hydrolysis	8
4.1.4	Photodegradation	8
4.1.4.1	Testing and Modeling Methodologies.....	8
4.1.4.2	Summary of Available Data	9
4.1.4.3	Data Assessment and Test Plan for Photodegradation	9
4.1.5	Fugacity Modeling	10
4.1.5.1	Modeling Methodologies	10
4.1.5.2	Summary of Available Data	10
4.1.5.3	Test Plan for Fugacity	10
4.2.	ECOTOXICOLOGY DATA	11
4.2.1	Aquatic Ecotoxicity Testing.....	11
4.2.1.1	Test Methodologies	11
4.2.1.2	Test Solution Preparation	12
4.2.1.3	Reporting Toxicity Results.....	13
4.2.2	Aquatic Toxicity of Members from the Alkenyl Succinic Anhydride Category	13
4.2.2.1	Summary of Available Data	14
4.2.2.1.1	Alga Toxicity	14
4.2.2.2	Data Assessment and Test Plan for Aquatic Toxicity	14
4.3	MAMMALIAN TOXICOLOGY DATA	14
4.3.1	Physicochemical Properties Relevant to Mammalian Toxicity	14
4.3.2	Acute Mammalian Toxicity of Members from the Alkenyl Succinic Anhydride Category	15
4.3.2.1	Acute Toxicity Test Methodology.....	15
4.3.2.2	Summary of Available Data	16
4.3.2.2.1	Acute Oral Toxicity	16
4.3.2.2.2	Acute Dermal Toxicity	16
4.3.2.2.3	Acute Inhalation Toxicity	16
4.3.2.3	Data Assessment and Test Plan for Acute Mammalian Toxicity	17
4.3.3	Mutagenicity of Members from the Alkenyl Succinic Anhydride Category	17
4.3.3.1	Mutagenicity Test Methodology	17
4.3.3.2	Summary of Mutagenicity Data	18
4.3.3.3	Data Assessment and Test Plan for Mutagenicity	18
4.3.4	Repeated-dose Toxicity of Alkenyl Succinic Anhydride Category.....	18
4.3.4.1	Repeated-dose Toxicity Test Methodology.....	18
4.3.4.2	Summary of Repeated-Dose Toxicity Data	19

Group 16 - Alkenyl Succinic Anhydride Category
November 22, 2002

TABLE 1. Members of the Alkenyl Succinic Anhydride Category	20
TABLE 2. Chemical Structures of Members of the Alkenyl Succinic Anhydride Category	21
TABLE 3. Selected Physicochemical Properties and Proposed Testing	22
TABLE 4. Environmental Fate Data and Proposed Testing	23
TABLE 5. Aquatic Toxicity Data and Proposed Testing	24
TABLE 6. Acute Mammalian Toxicity Data	25
TABLE 7. Mutagenicity Data and Proposed Testing	26
TABLE 8. Repeated-dose Mammalian Toxicity Data and Proposed Testing	26

1.0 INTRODUCTION

In March 1999, the American Chemistry Council (formerly the Chemical Manufacturers Association) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address data needs for certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address physico-chemical, environmental, aquatic and health effects testing information for the following three substances used as intermediates in the synthesis of petroleum additives:

- 2,5-Furandione, dihydro-3-(tetrapropenyl)-, (CAS #26544-38-7), referred to as "tetrapropenylsuccinic anhydride".
- Butanedioic acid, (tetrapropenyl)-, (CAS #27859-58-1), referred to as "tetrapropenyl butanedioic acid".
- 2,5-Furandione, 3-(dodecenyl) dihydro-, (CAS #25377-73-5), referred to as "dodecenylsuccinic anhydride".

Although the United States Toxic Substances Control Act - Inventory Update Rule (IUR) report lists 1990 production volumes for *straight-chain* C12 (i.e., dodecyl) succinic anhydride, additive manufacturers synthesize *branched* C12 (i.e., tetrapropenyl) succinic anhydride using propene tetramer. Although several studies identify the dodecyl derivative as the test substance, the tetrapropenyl derivative is and always has been the manufactured chemical. In this test plan, CAS #25377-73-5 and CAS #26544-38-7 are considered interchangeable and refer to the same substance, tetrapropenylsuccinic anhydride. It is also important to emphasize that tetrapropenylsuccinic anhydride (CAS #26544-38-7) can hydrolyze to tetrapropenyl butanedioic acid (CAS #27859-58-1). Because under many conditions the diacid form of the anhydride will be the form that exists and drives concern, consideration of both is necessary to adequately assess hazard for this category. For this reason, the anhydride and diacid are included together in this test plan.

EPA guidance on the HPV Chemical Challenge Program indicates that the primary purpose of the program is to encourage "the chemical industry . . . to voluntarily compile a Screening Information Data Set (SIDS) on all chemicals on the US HPV list." (EPA, "Development of Chemical Categories in the HPV Challenge Program," p. 1) At the same time, EPA recognizes that the "large number of chemicals to be tested [about 2800 HPV chemicals] makes it important to reduce the number of tests to be conducted, *where this is scientifically justifiable*." (*Id.*, p. 1) [emphasis added] The next part of the guidance explains where this would be scientifically justifiable:

One approach is to test closely related chemicals as a group, or category, rather than test them as individual chemicals. In the category approach, *not every chemical needs to be tested for every SIDS endpoint*. However, *the test data finally compiled* for the category

must prove adequate to support a screening level hazard-assessment of the category and its members. That is, the *final data set* must allow one to estimate the hazard for the untested endpoints, *ideally* by interpolation between and among the category members. In certain cases, where toxicity is low and no upward trend is expected, extrapolation to the higher category members may be acceptable. (*Id.*, p. 1) [emphasis added].

EPA guidance goes on to state, “The use of categories is encouraged in the Challenge Program and will have a number of benefits.” (*Id.*, p. 1) Among the benefits identified in the guidance for the use of categories are “a reduction in testing will result in fewer animals used to test a category of chemicals as opposed to doing each test on each individual chemical,” and “there will be . . . economic savings since less testing may be needed for chemicals considered as a category.” (*Id.*, p. 1) That guidance also states that categories “accomplish the goal of the Challenge Program – to obtain screening level hazard information – through the strategic application of testing to the category.” (*Id.*, p. 2)

A similarly stated intent “to reduce the number of tests to be conducted, *where this is scientifically justifiable*” was articulated by the Agency in its draft guidance document titled, “The Use of Structure Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program.” [emphasis added].

The EPA “Chemical Categories” guidance sets forth a definition of what constitutes a “chemical category, for the purposes of the Challenge Program”. Specifically, that definition states that a chemical category under the HPV Challenge Program “is a group of chemicals whose physicochemical and toxicological properties *are likely to* be similar *or* follow a regular pattern as a result of structural similarity.” (*Op. Cit.*, p. 2) [emphasis added].

According to the guidance, what is important is that the “structural similarities [among members of the group] *may* create a predictable pattern *in any* or all of the following parameters: physicochemical properties, environmental fate and effects, and human health effects.” (*Id.*, p. 2) [emphasis added]. Thus, it is not necessary for the chemicals in a category to be similar in all respects. Nor must there be conclusive proof that the chemicals in the postulated category will behave identically across all relevant parameters. All that is required for an acceptable category under the HPV Challenge Program is that there be a *likelihood* of similarity of physicochemical and toxicological properties or a *likelihood* that the chemicals will in some pertinent respect follow a regular pattern as a result of their structural similarity.

In identifying the alkenyl succinic anhydride category, the six-step process set out in the EPA guidance on category development was followed. As the information below indicates, the alkenyl succinic anhydride chemicals clearly satisfy the standards established in that guidance for use of a chemical category:

Step 1: group structurally similar chemicals into a putative category

Step 2: gather relevant published and unpublished literature for each member of the category

Step 3: evaluate the compiled data for adequacy in accordance with the EPA guidance documentation

Step 4: construct matrices of SIDS endpoints versus category members arranged so as to indicate the structural progression of the category (in this case, by increasing molecular weight)

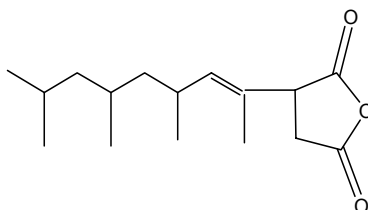
Step 5: evaluate the data to determine whether there is a correlation between category members for each SIDS endpoint

Step 6: make available to EPA, and to the public for review, this test plan including the foregoing category definition and rationale and the following data assessment with the proposed testing scheme for the alkenyl succinic anhydrides.

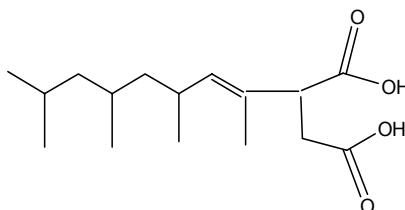
2.0 CHEMISTRY OF ALKENYL SUCCINIC ANHYDRIDES

2.1 DESCRIPTION

The alkenyl succinic anhydride consists of a tetrapropenyl moiety, a mono unsaturated branched hydrocarbon, and a succinic anhydride group. A general structure is shown below as CAS #26544-38-7:



26544-38-7



27859-58-1

The second member of this category is the diacid (CAS #27859-58-1) of the anhydride structure pictured above. The chemical names, simplified names, CAS numbers, and structures for the members of the alkenyl succinic anhydride category are presented in Tables 1 and 2. These substances are produced from the reaction of isomerized olefins and maleic anhydride at a temperature of 200°C. The maleic anhydride is generally added slowly over the course of the

reaction as the alkenylsuccinic anhydride helps solubilize the maleic anhydride. At the end of the reaction the olefin is stripped off and most unreacted maleic anhydride is also stripped off at the same time. The dark tarry poly maleic anhydride is usually filtered off. Adding the maleic anhydride over time helps minimize the polymaleic anhydride side reaction which is clearly undesirable. Most of the time, isomerized olefins are best since 1-dodecene is a solid, while the 2, 3 and 4-isomers are liquids. In most cases, additive manufacturers synthesize the *branched* C12 tetrapropenyl congener using propylene tetramer. Propylene tetramer is a distilled product manufactured from oligomerization of 1-propene under acid catalysis (e.g., polyphosphoric acid) conditions. Commercial propylene tetramer is a range of C10-C15 olefins with the C12 propylene tetramer isomer being ~60 wt-% of the total. Although study reports may identify the dodecenylsuccinic derivative as the test substance, the tetrapropenylsuccinic derivative is and always has been the manufacture chemical. The diacid is made by adding water to the alkenyl succinic anhydride and heating. Diacid is used as a corrosion inhibitor.

2.2 PHYSICOCHEMICAL PROPERTIES

Selected physicochemical properties of members from the alkenyl succinic anhydride category are presented in Table 3.

2.2.1 Molecular Weight

Members of the category have molecular weights of either 266.4 or 284.4 daltons (Table 3).

2.2.2 Specific Gravity

The specific gravity of category members is approximately 1.0 g/ml (@25°C) (Table 3).

2.2.3 Melting Point and Boiling Point

Alkenyl succinic anhydride and the diacid, as manufactured, are liquid at most ambient temperatures. Modeling data indicate that the melting point of these substances can range from approximately 14°C to 40°C for the anhydrides and 151°C for the diacid (Table 3). Modeling data indicate that the boiling point of these substances can range from approximately 334°C to 449°C for the anhydrides and 409°C for the diacid (Table 3).

2.2.4 Vapor Pressure and Viscosity

The low volatility of category members can be associated with their low vapor pressure, and high viscosity. Modeling data indicate that the vapor pressure of the members in category are equal to or less than 3E-4 Pa @ 25 °C (see Table 3). The viscosity of dodecenylsuccinic anhydride (CAS #25377-73-5) is measured as 200 cSt @ 25°C (Table 3).

2.2.5 Water Solubility

The alkenylsuccinic anhydrides hydrolyze to alkenyl butanedioic acids in aqueous solution. The water solubility of the tetrapropenyl butanedioic acid (CAS #27859-58-1) was calculated as 3.2 mg/L (see Table 3). This value indicates that the diacid of the anhydride members of this category are sparingly soluble in water. This value will be confirmed by developing measured water solubility data for this substance.

2.2.6 Octanol-Water Partition Coefficient

The log octanol-water partition coefficient (Kow) value of the tetrapropenyl butanedioic acid (CAS #27859-58-1) is calculated as 4.8 (see Table 3). Kow values for the anhydrides are not provided because these substances would not be present in their anhydride forms in the aqueous phase.

3.0 USES OF THE ALKENYL SUCCINIC ANHYDRIDE CATEGORY

Alkenyl succinic anhydrides are intermediates used in the synthesis of corrosion inhibitor components in lubricants (e.g., motor oils, metalworking oils, industrial oils) by the petroleum additive industry. Other non-petroleum additive applications of alkenyl succinic anhydrides includes the following: a) intermediates in the production of surfactants, b) epoxy curing agents, c) leather tanning agents, and d) paper sizing agents. These non-petroleum additive applications will not be discussed in this test plan.

Alkenyl succinic anhydrides are manufactured and blended into additive packages at plants owned by members of the HERTG. They are generally sold to finished oil blenders in additive packages, where the concentration ranges from 0.12 to 1.0 wt-%. These additive packages are then blended into finished products where the typical concentration of alkenyl succinic anhydrides ranges from 0.1 to 1.0 ppm. Finished lubricants are blended at facilities owned by our customers. Additive packages are shipped to customers in bulk using ships, isocontainers, railroad tank cars, tank trucks, or 55-gallon steel drums. The anhydride products are carefully protected from moisture during transportation and storage to avoid hydrolysis to the diacid.

Bulk additive packages are stored in bulk storage tanks at the customer blending sites. Finished oils are blended by pumping the lubricating oil blend stocks and the additive package from their storage tanks through computer controlled valves that meter the precise delivery of the components into a blending tank. After blending, the finished lubricant products are sold in bulk and shipped in tank trucks to large industrial users, such as manufacturing facilities and facilities that service truck fleets and passenger motor vehicles. Finished lubricants are also packaged into 55-gallon drums, 5-gallon pails, and one-gallon and one-quart containers for sale to smaller industrial users. Sales of lubricants in one-gallon and one-quart containers to consumers at service stations or retail specialty stores also occur.

Based on these uses, the potentially exposed populations include (1) workers involved in the manufacture of alkenyl succinic anhydrides, synthesis of components, the blending of additive packages, and blending the additive packages into finished lubricants; (2) quality assurance workers who sample and analyze these products to ensure that they meet specifications; (3) workers involved in the transfer and transport of alkenyl succinic anhydrides, additive packages or finished lubricants that contain them; (4) mechanics who may come into contact with both fresh and used lubricants while working on engines or equipment; (5) gasoline station attendants and consumers who may periodically add lubricating oil to automotive crankcases; and (6) consumers who may change their own automotive engine oil.

The most likely route of exposure for these substances is skin and eye contact. Manufacturing, quality assurance, and transportation workers will likely have access to engineering controls and wear protective clothing to eliminate exposure. Mechanics wear protective clothing, but often work without gloves or eye protection. Gasoline station attendants and consumers often work without gloves or other protective equipment. The most likely source of environmental exposure is accidental spills at manufacturing sites and during transport.

4.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA

4.1 ENVIRONMENTAL FATE DATA

4.1.1 Physicochemical Properties Relevant to Environmental Fate

In order to evaluate the environmental fate of a substance, it is important to understand its potential degradability and partitioning behavior among environmental compartments (i.e., air, soil, sediment, suspended sediment, water, and biota).

The physicochemical properties and molecular structure of a chemical will influence the degradation processes it may be subjected to in the environment. Potentially important environmental degradation pathways include biodegradation, hydrolysis, and photodegradation. Biodegradation of an organic chemical by bacteria can provide energy and carbon for microbial growth. This process results in a structural change of the chemical. Biodegradation can result in the complete loss of an organic chemical, producing carbon dioxide, mineral salts, and water. Hydrolysis is a reaction in which a water molecule or hydroxide ion substitutes for another atom or group of atoms present in an organic chemical resulting in a structural change of that chemical. Chemical photodegradation results in a structural change of a molecule from the absorption of solar radiation.

The physicochemical properties of a substance will also influence the way in which it partitions among environmental compartments. Generally, substances characterized by a low vapor pressure do not partition into air to any great extent.

Similarly, substances that are characterized by a lower water solubility do not partition extensively into water. Substances that do not partition into air and water to any great extent tend to partition into soil and sediments.

4.1.2 Biodegradation

4.1.2.1 Test Methodologies

The potential biodegradability of a substance in water, under aerobic conditions can be assessed using one of the OECD 301 testing guidelines. Chemical biodegradation involves a series of microbial-mediated reactions that can require many kinds of microorganisms acting together to degrade the parent substance. There are several standard test methods, which measure primary degradation (i.e., loss of parent chemical) or ultimate degradation (i.e., complete utilization of the substance to produce carbon dioxide, water, mineral salts, and microbial biomass). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals, infrared absorbance, or by a chemical-specific detection method. Ultimate degradation (also called mineralization) can be determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on an elemental analysis of the chemical under investigation.

4.1.2.2 Summary of Available Data

Biodegradation data for the alkenyl succinic anhydride category are summarized in Table 4.

Since dodecenylsuccinic anhydride and tetrapropenylsuccinic anhydride hydrolyze to their alkenyl butanedioic acids, using the biodegradability of the diacid to assess the biodegradability of the anhydrides is appropriate. Tetrapropenyl butanedioic acid (CAS #27859-58-1) was evaluated using the Manometric Respirometry Test (OECD Guideline 301F). After 28-days, this substance exhibited 18.3% biodegradation, based on theoretical oxygen demand.

4.1.2.3 Data Assessment and Test Plan for Biodegradability

Adequate biodegradation data exist for tetrapropenyl butanedioic acid (CAS #27859-58-1). Since the alkyl side chains of substances in this category are predominantly branched, the results indicate that these substances would exhibit limited biodegradation under the conditions of the test system. These results will be used to bridge to all category members, thereby characterizing the biodegradability of the entire category.

4.1.3 Hydrolysis

4.1.3.1 Test Methodologies

The potential for a substance to hydrolyze in water can be assessed as a function of pH (OECD Guideline 111, *Hydrolysis as a Function of pH*¹). When an organic molecule undergoes hydrolysis, a nucleophile (water or hydroxide ion) attacks an electrophile and displaces a leaving group (e.g., halogen, phenoxide)². Potentially hydrolyzable groups include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters³, as well as anhydrides. Otherwise, the lack of a suitable leaving group renders compounds resistant to hydrolysis.

4.1.3.2 Summary of Available Data

There are no published or unpublished hydrolysis studies for members of this category.

4.1.3.3 Data Assessment and Test Plan for Hydrolysis

Tetrapropenylsuccinic anhydride contains a functional group that has the potential to hydrolyze. Alkenyl butanedioic acid is the hydrolysis product of this anhydride. This reaction is believed to occur at a rapid rate. The public and private literature will be reviewed to identify if there is sufficient information that can be used to assess the potential hydrolysis rate of the alkenyl succinic anhydride. In the event that there are insufficient information, the hydrolysis rate of tetrapropenyl butanedioic acid (CAS #27859-58-1) will be evaluated using the testing method described above.

4.1.4 Photodegradation

4.1.4.1 Testing and Modeling Methodologies

Photodegradation can occur as a result of direct and indirect mechanisms. Direct photodegradation can be measured in solution using the OECD test guideline 113, while indirect photodegradation can be estimated using a model accepted by the US EPA.

Simple chemical structures can also be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be

¹ Organization for Economic Cooperation and Development (OECD) (1993) OECD Guidelines for Testing of Chemicals. OECD. Paris, France.

² W. Lyman et al. (1990) Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co. New York, NY, USA.

³ W. Lyman et al. (1990) Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co. New York, NY, USA.

calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline⁴.

An estimation method for indirect photodegradation that is accepted by the US EPA applies a calculation procedure to determine an atmospheric oxidation potential (AOP) value. The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows) (EPIWIN, 1999) is used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances) to estimate AOP values. This program calculates a chemical half-life based on an overall OH- reaction rate constant, a 12-hr day, and a given OH- concentration.

4.1.4.2 Summary of Available Data

Published or unpublished photodegradation studies and AOP data for members of the alkenyl succinic anhydride category are not available.

4.1.4.3 Data Assessment and Test Plan for Photodegradation

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may lead to its transformation. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. Indirect photodegradation also requires light energy as well as a series of chemical reactions that include a reaction of the parent molecule with hydroxyl radicals (OH-).

An initial review of the members of the alkenyl succinic anhydride category suggests that the members do not contain bonds that have a high potential to absorb UV light above 290 nm. Further, these substances have low vapor pressure, which indicates that they have a low potential to partition into the air to a significant extent where they would be subject to indirect photodegradation.

To develop adequate data for this endpoint, the UV light absorptive potential of chemicals in this category will be evaluated to identify those chemicals with a potential to degrade in solution. When possible, first order reaction rates will be calculated for chemicals identified to have a potential for direct photolysis in water. The results of the calculations will be summarized in a technical discussion in the form of a robust summary. If instead, a low potential for direct photolysis is suggested by the evaluation, a technical discussion will be prepared as a robust summary describing the findings.

⁴ Zepp, R. G., and D. M. Cline. 1977. Rates of Direct Photolysis in the Aqueous Environment. *Environ. Sci. Technol.* 11:359.366.

The AOP data for representative structures of the category (Table 2) will be estimated and the following data provided in a robust summary:

- Rate constants for the atmospheric, gas phase reaction as mediated by photochemically produced hydroxyl radicals.
- Atmospheric half-lives based on hydroxyl radical attack.

4.1.5 Fugacity Modeling

4.1.5.1 Modeling Methodologies

Fugacity-based multimedia fate modeling calculates the relative distribution of a chemical between environmental compartments. A widely used model for this approach is the EQC model⁵.

There are multiple levels of the EQC model, which vary in complexity and data requirements. In the document, "Determining the Adequacy of Existing Data", EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. The EQC Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized environment (unit world). Another EQC model, the Level III model, uses these parameters, as well as chemical emission rates into air, water, and soil, and chemical degradation rates in air, water, soil, and sediment. Because much of this information is not available and because using default values could develop incorrect data, Level I partitioning data will be developed for members of this category.

4.1.5.2 Summary of Available Data

There are no published or unpublished fugacity-based multimedia fate modeling data for members of the alkenyl succinic anhydride category. All of the members of this category have low vapor pressure and sparingly low water solubility suggesting that they will not tend to partition into the air or water to any great extent.

4.1.5.3 Test Plan for Fugacity

The relative distribution of substances within this category among environmental compartments will be evaluated using the Level I model. Data developed using a Level I model can then be used for simple comparative purposes across several substances. EQC Level III will not be used for this evaluation because appropriate emission levels are as yet unknown. Because of the physical nature of the substances in this category, a Level I data set will be as equally robust as a Level

⁵ Mackay, D., A. Di Guardo, S. Paterson, and C. E. Cowan. 1996. Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. *Environ. Tox. Chem.* 15:1627-1637.

III data set and can then be used to assess the potential partitioning behavior of the category members in the environment.

Input data to run the EQC Level I model may require an additional computer model to estimate selected physical/chemical properties from a structure. The model used for this purpose will be EPIWIN, version 3.04⁶, which was developed by the Syracuse Research Corporation. EPIWIN includes algorithms for estimating all physical and chemical properties needed for the EQC model. The representative structures that will be used are listed in Table 2.

4.2. ECOTOXICOLOGY DATA

4.2.1 Aquatic Ecotoxicity Testing

4.2.1.1 Test Methodologies

Acute aquatic ecotoxicity testing can include three species that represent three trophic levels in the freshwater aquatic environment: fish, invertebrates, and algae. The fish acute toxicity test (OECD Guideline 203, *Fish, Acute Toxicity Test*) determines the lethality of a substance to a fish during a 96-hour exposure period. The invertebrate acute toxicity test (OECD Guideline 202, *Daphnia sp., Acute Immobilization Test and Reproduction Test*) determines the potential of a substance to immobilize an invertebrate, typically a daphnid (*Daphnia magna*), during a 48-hour exposure period. The alga growth inhibition test (OECD Guideline 201, *Alga, Growth Inhibition Test*) determines the potential of a substance to inhibit alga growth, typically using the freshwater unicellular green algae, *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*), during a 72- or 96-hour exposure period.

Three different exposure methodologies are available to conduct aquatic toxicity tests; i.e., flow-through, static, and static renewal.

In *flow-through exposures*, organisms are exposed to a constant chemical concentration or loading in each treatment level in the incoming water and there is typically greater assurance than with other test methods that the exposure levels and water quality remains constant throughout the test. Although flow-through testing is the preferred method, it is most applicable for chemicals that have adequate water solubility for testing.

In *static exposures*, organisms are exposed to a chemical in a test medium that is not replaced for the duration of the study. There is less assurance that the test concentrations or loadings to which test organisms are exposed will remain constant because test substance can be adsorbed onto test chambers, degraded,

⁶ EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.

volatilized, or otherwise changed during the test. Nevertheless, due to limitations of other test systems for non-volatile substances, the static test has been widely used and in some instances must be used, as is the case when conducting an alga test.

The *static-renewal exposure* is similar to a static exposure because it is conducted in still water, but the test solutions and control water are renewed periodically, usually every 24 hours. Daily test solution renewal provides a greater likelihood that the exposure concentrations or loadings will remain stable throughout the test. This is the preferred exposure method for conducting fish toxicity tests for compounds in this category. Daily renewals cannot be performed in the alga test because the process of exposure solution separation and replenishment can cause a discontinuity in the alga growth rate. Also, dependent on the substance and test procedure used, renewals may not be possible for the *Daphnia* test because the procedure can stress *Daphnia* or result in coating or entrapping the organisms in surface film that may form during renewal operations. OECD considers the use of static testing for fish, *Daphnia*, and algae, and the use of static renewal testing for fish to be appropriate when evaluating the toxicity of sparingly water-soluble substances like those in this category provided that test solution preparation uses water accommodated fraction or water soluble fraction methods.⁷

4.2.1.2 Test Solution Preparation

Alkenyl succinic anhydrides are sparingly soluble to insoluble in water, and it is not possible to prepare exposure solutions for aquatic toxicity testing by direct addition of measured quantities of test material to water. Two methods⁸ are used to prepare solutions of poorly water-soluble materials for aquatic toxicity testing:

- *Water accommodated fraction (WAF)* – This is a method in which the test solution contains only that fraction of the test material (organic phase) which is retained in the aqueous phase after a period of stirring long enough to reach equilibrium, followed by a sufficient time (1-4 hours) for phase separation. The WAF (aqueous phase) will contain soluble components of the test material at levels that will be dependent on the test material loading (the amount of material added to the aqueous medium). The resulting WAF is used in the aquatic toxicity test. Ideally, a WAF consists of a water-soluble extract of test material, but it can also include a stable micro-emulsion or contain small amounts of suspended matter.
- *Water soluble fraction (WSF)* – This is a method in which a WAF is either filtered, centrifuged, or allowed to settle for a greater length of time (24 hours)

⁷ Organization for Economic Cooperation and Development (OECD) (2000). Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No.23, Paris, France.

⁸ American Society for Testing and Materials (1998) D6081-98, Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation.

than with the WAF method to remove suspended matter from the aqueous phase before being used in the aquatic toxicity test.

4.2.1.3 Reporting Toxicity Results

In both WAF and WSF tests, test material concentrations are expressed as loading rates (i.e., defined as the weight of test material added per unit volume of test medium during WAF or WSF preparation)⁹. For fish tests, endpoints can be expressed as median lethal loading rate (LL₅₀) when lethal effects occur to 50% of the test population or in cases where no lethal effects are observed at all loadings tested, LL₀. In both cases, results can be expressed in mg/L and in studies where no lethality is observed, the result is expressed as LL₀ = the highest loading rate tested. For invertebrate and alga tests, endpoints are expressed as median effective loading rate (EL₅₀) or EL₀ in mg/L as discussed above.

Loading rates allow sparingly water-soluble to insoluble complex substances such as the alkenyl succinic anhydrides to be compared to more readily soluble substances and/or pure chemicals on an equal basis. To allow comparison, the toxicity value is expressed as the amount of test material added per unit volume of water when preparing the WAF or WSF.

If test material exposure levels are analytically measured in the test, the endpoints can also be expressed as median lethal concentration (LC₅₀) or median effective concentration (EC₅₀) in mg/L. EC/LC₅₀s are often not reported because it is very difficult to accurately measure test material exposure levels that can be below 1.0 mg/L.

NOTE: In this test plan, these results are reported as loading rates (EL/LL), to reflect the current reporting practices for the WAF method used in the tests. In the robust summaries, these data are presented as concentrations (EC/LC) as originally reported even though the test methods employed WAF preparation of test solutions without measurement of test material concentration.

4.2.2 Aquatic Toxicity of Members from the Alkenyl Succinic Anhydride Category

In general, the toxicity of a substance to an organism is limited by mechanisms of uptake and movement to target organs. Characteristics such as smaller molecular size and a lesser degree of ionization increase the ability of a substance to passively cross biological membranes. However, the soluble fraction of a compound in water represents the chemical fraction responsible for toxicity to

⁹ Organization for Economic Cooperation and Development (OECD) (1999) Draft Guidance document on Aquatic Toxicity Testing of Difficult Substances. OECD, France.

aquatic organisms. Therefore, aquatic toxicity can be limited by the water solubility of a substance.

Preliminary modeling information indicates that members of the alkenyl succinic anhydride category have sparingly low water solubility. This assessment is based on the diacid form of the anhydrides. The diacid form is used because aquatic organisms will only be exposed to the diacid, which is the hydrolyzed form of the anhydride. The length of the alkenyl side chain on these substances will influence their relative water solubility, and thus possibly, their relative toxicity.

4.2.2.1 Summary of Available Data

Acute aquatic ecotoxicity data for the alkenyl succinic anhydride category is summarized in Table 5. One member (i.e., tetrapropenyl butanedioic acid) of the category has been tested for effects on algal growth. There are no other reliable aquatic toxicity data for members of the alkenyl succinic anhydride category.

4.2.2.1.1 Alga Toxicity

Tetrapropenyl butanedioic acid (CAS #27859-58-1) was evaluated for effects on the growth of the unicellular green alga, *Pseudokirchneriella subcapitata*, in a 96-hour exposure. The test substance loading rate were from 0.3 to 3,000 mg/L. The test substance was considered algistatic to freshwater alga, at loading rates of 330 and 3000 mg/L. The EC₅₀ for this substance was 93 mg/L.

4.2.2.2 Data Assessment and Test Plan for Aquatic Toxicity

As previously mentioned, although dodecenylsuccinic anhydride (CAS #26377-73-5) is listed as an HPV chemical, additive manufacturers typically synthesize branched C12 (i.e., tetrapropenyl) alkenyl succinic anhydrides using propylene tetramer. Further, in the aquatic environment, tetrapropenylsuccinic anhydride (CAS #26544-38-7) will hydrolyze to tetrapropenyl butanedioic acid (CAS #27859-58-1). Therefore, tetrapropenyl butanedioic acid (CAS #27859-58-1) will be evaluated for toxicity to a fish and invertebrate. These data will be used to bridge to the two anhydride members of this category to characterize their aquatic toxicity.

4.3 MAMMALIAN TOXICOLOGY DATA

4.3.1 Physicochemical Properties Relevant to Mammalian Toxicity

Physicochemical properties of chemicals are useful for predicting the routes by which exposure may occur, and in some cases, the mechanism and extent of

toxicological responses. The physicochemical properties of the alkenyl succinic anhydride are presented in Table 3. These lubricant additive intermediates are liquid substances with low octanol/water partition coefficients and sparingly water solubilities. These characteristics indicate that alkenyl succinic anhydrides are slightly lipophilic, and thus, capable of passive diffusion across biological membranes. It would be predicted that upon oral exposure these chemical substances would be absorbed by the gastrointestinal tract. However, the structural and physical properties such as comparatively high molecular weight, the presence of long-chain tetrapropenyl moieties and sparingly water solubility, is expected to impede the rate and extent of absorption of alkenyl succinic anhydride from dermal surfaces. In addition to the general considerations discussed above, the alkenyl succinic anhydrides have relatively high boiling points, low vapor pressure, and are viscous liquids. As a result, these substances have a low propensity to form vapors or aerosols, and thus, unintentional exposure via inhalation is uncommon.

4.3.2 Acute Mammalian Toxicity of Members from the Alkenyl Succinic Anhydride Category

4.3.2.1 Acute Toxicity Test Methodology

Acute toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. Potential routes of exposure for acute toxicity assays include oral, dermal, and inhalation. Oral toxicity assays are conducted by administering test material to fasted animals (typically rats or mice) in a single gavage dose. Acute dermal toxicity tests are conducted by administering test material to the shaved skin on the back of the test animal (typically rats or rabbits) and allowing the test material to stay in contact with the skin application site for a specific duration (usually 24 hours). Acute inhalation toxicity assays are conducted by exposing test animals (typically rats) in a controlled atmosphere to a fixed air concentration of the test substance for a specific duration (typically 4 hours). The test material is either generated as a vapor or intentionally aerosolized into respirable particles, then metered into the exposure air at the desired concentration. Preferably, inhalation toxicity studies are conducted using either nose-only or head-only exposure to minimize potential confounding effects resulting from whole-body exposure. Whole body exposure may lead to over-prediction of inhalation toxicity hazard by increasing the body-burden of the test material through skin absorption or ingestion of test material as a consequence of grooming both during and after the inhalation exposure period.

Historically, lethality is a primary end-point of concern in acute toxicity studies, and the traditional index of oral and dermal potency is the median lethal dose that causes mortality in 50 percent of the test animals (LD₅₀). In acute inhalation studies, the traditional measurement of potency is the median lethal concentration of the test material in air that causes mortality in 50 percent of the test animals (LC₅₀). In addition to lethality, acute toxicity studies also provide insights

regarding potential systemic toxicity through careful observation and recording of clinical signs and symptoms of toxicity as well as through detailed examination of tissues and organ systems.

Typically, acute oral and dermal toxicity studies are conducted using a limit dose of 5000 and 2000 mg/kg body weight, respectively, and acute inhalation toxicity studies are conducted using a limit dose of 5 mg/L for 4 hours (according to OECD and EPA testing guidelines). Prior to 1990, some acute dermal toxicity studies may have used a limit dose of 5000 mg/kg. Recently, harmonized EPA testing guidelines (August 1998) have set the limit dose for both oral and dermal acute toxicity studies at 2000 mg/kg body weight, while the recommended limit concentration for acute inhalation studies has been set at 2 mg/L for 4 hours. The limit dose test method minimizes the number of animals tested by exposing a single group of animals to a large dose (the limit dose) of the test substance. A test substance that shows little or no effects at the limit dose is considered essentially nontoxic, and no further testing is needed. If compound-related mortality is observed at the limit dose, then further testing may be necessary.

4.3.2.2 Summary of Available Data

Acute toxicity data for the alkenyl succinic anhydride category is summarized in Table 6. Two of the three members of the category have been tested by the oral and dermal route of administration and demonstrated a low order of acute toxicity.

4.3.2.2.1 Acute Oral Toxicity

Two substances in the alkenyl succinic anhydride category have been adequately tested for acute oral toxicity. The acute oral LD₅₀ for these studies in rats is greater than 2000 mg/kg and indicative of a relatively low order of acute toxicity.

4.3.2.2.2 Acute Dermal Toxicity

One substance in the alkenyl succinic anhydride category has been adequately tested for acute dermal toxicity. The acute dermal LD₅₀ for this study in rabbits was greater than 5000 mg/kg and indicative of a relatively low order of acute toxicity.

4.3.2.2.3 Acute Inhalation Toxicity

Dodecenylsuccinic anhydride (CAS #25377-73-5) has been adequately tested for acute inhalation toxicity¹⁰. The acute inhalation LCLo for this study in rats

¹⁰R.J. Lewis (1998), Sax's Dangerous Properties of Industrial Materials, 9th Edition, John Wiley & Sons, Inc., New York, NY, USA.

was 1220 mg/m³/4 hours and indicative of a relatively low order of acute toxicity.

4.3.2.3 Data Assessment and Test Plan for Acute Mammalian Toxicity

In total, three adequate acute toxicity studies have been conducted for two members of the alkenyl succinic anhydride category. These studies involved two species of laboratory animals (rats and rabbits); two routes of exposure (oral and dermal), and evaluated the toxicity of two members of the category. The data consistently demonstrate a low order of acute toxicity.

Based on the results of these studies, the acute toxicity of the category has been evaluated adequately with respect to all acute toxicity endpoints, and no additional acute toxicity testing is proposed for the HPV Challenge Program.

4.3.3 Mutagenicity of Members from the Alkenyl Succinic Anhydride Category

4.3.3.1 Mutagenicity Test Methodology

Genetic toxicology is concerned with the effects of substances on genetic material (i.e., DNA and chromosomes). Within genetic material, the gene is the simplest functional unit composed of DNA. Mutations are generally non-lethal, heritable changes to genes that may arise spontaneously or because of xenobiotic exposure. Genetic mutations are commonly measured in bacterial and mammalian cells. The simplest test systems measure the occurrence of a base-pair substitution mutation in which a single nucleotide is changed followed by a subsequent change in the complementary nucleotide on the other DNA strand. Frame shift mutations occur following the deletion or insertion of one or more nucleotides, which then changes the "reading frame" for the remainder of the gene or multiple genes. Genetic testing for these types of point mutations is generally accomplished by *in vitro* cellular assays for forward or reverse mutations. A forward mutation occurs when there is a detectable change in native DNA whereas a reverse mutation occurs when a mutated cell is returned to its initial phenotype. Both base-pair substitutions and frame shift mutations are routinely measured in bacterial cells by measuring the ability of a cell to acquire the capability to grow in an environment missing an essential amino acid. In these tests, a large number of cells are examined to demonstrate a significant increase in the frequencies of mutations that occur over the frequency of spontaneous mutations.

Chromosomal aberrations are large scale numerical or structural alterations in eukaryotic chromosomes including deletions (visualized as breaks), translocations (exchanges), non-disjunction (aneuploidy), and mitotic recombination. Chromosomal breakage is the classical end point in chromosomal aberration assays. Substances that induce structural changes in chromosomes, especially chromosome breaks, are referred to as "clastogens." To visualize chromosomes

and chromosomal aberrations following *in vitro* or *in vivo* treatment with a substance, cells are arrested in metaphase, treated to swell the chromosomes, fixed, transferred to slides and stained. The first metaphase following treatment is the time at which the greatest number of cells with damaged chromosomes may be observed. The most frequently used test systems investigate changes in mammalian cells (such as Chinese hamster ovary or lung cells; human or rat lymphocytes; or human, rat or mouse bone marrow cells) following either *in vitro* or *in vivo* exposure to the test substance.

4.3.3.2 Summary of Mutagenicity Data

A summary of the mutagenicity information for the alkenyl succinic anhydride category is presented in Table 7. *In vitro* bacterial gene mutation assays and *in vitro* chromosomal aberration assays have not been conducted for the three members of this category.

4.3.3.3 Data Assessment and Test Plan for Mutagenicity

Based on the propensity of anhydrides to hydrolyze under aqueous conditions, the tetrapropenyl butanedioic acid derivative (CAS #27859-58-1) will be tested and the data bridged to other members in category which lacks bacterial gene mutation and *in vitro* chromosomal aberration data for the HPV Challenge Program.

4.3.4 Repeated-dose Toxicity of Alkenyl Succinic Anhydride Category

4.3.4.1 Repeated-dose Toxicity Test Methodology

Repeated-dose toxicity studies evaluate the systemic effects of repeated exposure to a chemical over a significant period of the life span of an animal (rats, rabbits, or mice). Chronic repeated-dose toxicity studies are concerned with potential adverse effects upon exposure over the greater part of an organism's life span (e.g., one to two years in rodents). Subchronic repeated-dose studies are also concerned with effects caused by exposure for an extended period, but not one that constitutes a significant portion of the expected life span. Subchronic studies are useful in identifying target organ(s), and they can be used in selecting dose levels for longer-term studies. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of at least 28 days or up to 90 days (i.e., 4 to 13 weeks). A recovery period of two to four weeks (generally included in most study designs) following completion of the dosing or exposure period provides information on whether or not the effects seen during the exposure period are reversible upon cessation of treatment. The dose levels evaluated in repeated-dose toxicity studies are notably lower than the relatively high limit doses used in acute toxicity studies. The NOAEL (no observed adverse effect level), usually expressed in mg/kg/day, defines the dose of test material that produces no significant toxicological effects. If the test

material produces toxicity at the lowest dose tested (i.e., there is no defined NOAEL), the lowest dose that produced an adverse effect is defined as the LOAEL (lowest observed adverse effect level). While these studies are designed to assess systemic toxicity, the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

Reproductive and developmental toxicity studies generate information on the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, and development of the conceptus, parturition, and post-partum development of the offspring. Various study designs exist, but they all involve exposure to both male and female test animals before mating. The rat is most often selected as the test species. The test substance is administered to males and females continuously at several graduated doses for at least two weeks prior to mating and until the animals are sacrificed. The males are treated for at least two more weeks. Male gonadal histopathology is carefully assessed at the end of the study. The females are treated through parturition and early lactation. The adult females and offspring are typically studied until termination on post-natal day 21, or sometimes earlier. In addition to providing data on fertility and reproduction, this study design provides information on potential developmental toxicity following prenatal and limited post-natal exposure to the test substance. An NOAEL or LOAEL is also used to describe the results of these tests, with the exception that these values are derived from effects specific to reproduction or development.

The “toxicity to reproduction” requirement in the HPV Challenge Program can be met by conducting the *Reproduction/Developmental Toxicity Screening Test* (OECD Guideline 421) or by adding this screening test to a repeated-dose study (OECD Guideline 422, *Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test*). The *One-Generation Reproduction Toxicity Study* (OECD Guideline 415) is a more comprehensive protocol for the study of the effect of a test material on reproduction and development that also meets the OECD SIDS and the HPV Challenge Program requirements.

4.3.4.2 Summary of Repeated-Dose Toxicity Data

None of the members from the alkenyl succinic anhydride category have been tested for repeated-dose or reproductive and development toxicity.

Based on the propensity of anhydrides to hydrolyze under aqueous conditions, the tetrapropenyl butanedioic acid derivative (CAS #27859-58-1) will be tested and the data bridged to other members in category which lacks repeated-dose toxicity and reproductive/developmental toxicity data for the HPV Challenge Program.

TABLE 1. Members of the Alkenyl Succinic Anhydride Category

CAS Number	Chemical Name	Simplified Chemical Name
26544-38-7	2,5-Furandione, dihydro-3-(tetrapropenyl)-, derivatives	Tetrapropenylsuccinic anhydride
27859-58-1	Butanedioic acid, (tetrapropenyl)-, derivatives	Tetrapropenyl butanedioic acid
25377-73-5	2,5-Furandione, 3-(dodecenyl) dihydro-, derivatives	Dodecenylsuccinic anhydride

TABLE 2. Chemical Structures of Members of the Alkenyl Succinic Anhydride Category

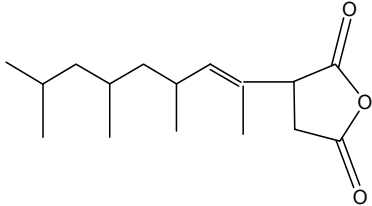
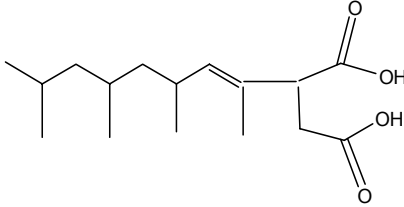
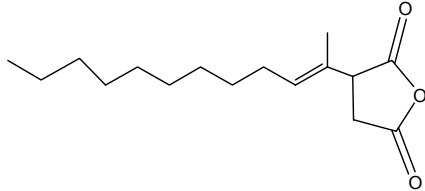
CAS Number	Chemical Structure
26544-38-7	 <p>26544-38-7</p>
27859-58-1	 <p>27859-58-1</p>
25377-73-5	 <p>25377-73-5</p> <p>Dodecenylsuccinic Anhydride</p>

TABLE 3. Selected Physicochemical Properties and Proposed Testing for Members of the Alkenyl Succinic Anhydrides Category

CAS Number	Molecular Weight	Specific Gravity¹ (g/ml)	Viscosity² (cSt @ 25°C)	Melting Point³ (°C)	Boiling Point⁴ (°C)	Vapor Pressure⁵ (Pa)	Water Solubility⁶ (mg/L)	Log Kow⁶
26544-38-7	266.4	1.005	ND	13.9	334.4	3E-4	NA ⁷	NA ⁸
27859-58-1	284.4	ND ¹²	ND	151.0	409.1	2.6E-7	Test (3.2 ⁹)	4.8
25377-73-5	266.4	1.002	200	40.3	348.8	1E-4	NA ¹⁰	NA ¹¹

¹ ASTM D1298-99, Standard Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method.

² ASTM D 445-97, Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (the Calculation of Dynamic Viscosity).

³ Modeling data; melting point is estimated (MPBWIN v1.40) and cannot be measured due to viscosity of liquid.

⁴ Modeling data; boiling point is estimated (MPBWIN v1.40) and cannot be measured because these substances decompose before they boil.

⁵ Modeling data; vapor pressure is estimated (MPBPWIN v1.40).

⁶ EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.

⁷ Not applicable; anhydrides form diacids in aqueous solutions, see CAS #27859-58-1 for water solubility of the diacid.

⁸ Not applicable; anhydrides form diacids in aqueous solutions, see CAS #27859-58-1 for the calculated Log Kow value of the diacid.

⁹ Modeling data; water solubility is estimated (KOWWIN v1.65).

¹⁰ Not applicable; anhydrides form diacids in aqueous solutions, the water solubility of the diacid of CAS #25377-73-5 is calculated to be 2.4 mg/L.

¹¹ Not applicable; anhydrides form diacids in aqueous solutions.

¹² No data needed (ND); bridging from other members of the category.

**TABLE 4. Environmental Fate Data and Proposed Testing for Members of the
Alkenyl Succinic Anhydrides Category**

CAS Number	BIODEGRADATION	HYDROLYSIS	PHOTODEGRADATION	FUGACITY
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
26544-38-7	No testing proposed Bridging	Technical discussion	Direct photodegradation evaluation AOPWIN model estimation	EQC model estimation
27859-58-1	18.3% biodegraded after 28-days	Technical discussion	Direct photodegradation evaluation AOPWIN model estimation	EQC model estimation
25377-73-5	No testing proposed Bridging	Technical discussion	Direct photodegradation evaluation AOPWIN model estimation	EQC model estimation

**TABLE 5. Aquatic Toxicity Data and Proposed Testing for Members of the
Alkenyl Succinic Anhydrides Category**

CAS Number	ACUTE TOXICITY TO FISH 96-hr LC ₅₀ (mg/L)	ACUTE TOXICITY TO INVERTEBRATES 48-hr EC ₅₀ (mg/L)	TOXICITY TO ALGAE 96-hr EC ₅₀ (mg/L)
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
26544-38-7	No testing proposed Bridging	No testing proposed Bridging	No testing proposed Bridging
27859-58-1	Test	Test	EC ₅₀ = 93 mg/L (WAF, PK)
25377-73-5	No testing proposed Bridging	No testing proposed Bridging	No testing proposed Bridging

WAF = Water Accommodated Fraction
PK = *Pseudokirchneriella subcapitata*

**TABLE 6. Acute Mammalian Toxicity Data for Members of the
Alkenyl Succinic Anhydride Category**

CAS Number	ACUTE ORAL TOXICITY¹	ACUTE DERMAL TOXICITY¹
	Available Data	Available Data
26544-38-7	LD ₅₀ > 2.0 g/kg (rat) ²	LD ₅₀ > 5.0 g/kg (rabbit) ²
27859-58-1	No testing proposed bridging	No testing proposed bridging
25377-73-5	LD ₅₀ > 2.0 g/kg (rat)	No testing proposed bridging

¹Toxicity endpoints are expressed as median lethal dose (LD₅₀) for acute oral and dermal toxicity.

² R.J. Lewis (1998). Sax's Dangerous Properties of Industrial Materials, 9th Edition, John Wiley & Sons, Inc., New York, NY, USA.

TABLE 7. Mutagenicity Data and Proposed Testing for Members of the Alkenyl Succinic Anhydride Category

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	Available Data & Proposed Testing
26544-38-7	No testing proposed bridging	No testing proposed bridging
27859-58-1	Test	Test
25377-73-5	No testing proposed bridging	No testing proposed bridging

TABLE 8. Repeated-dose Mammalian Toxicity Data and Proposed Testing for Members of the Alkenyl Succinic Anhydride Category

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
26544-38-7	No testing proposed bridging	No testing proposed bridging
27859-58-1	Test	Test
25377-73-5	No testing proposed bridging	No testing proposed bridging

CAS Number	Environmental Fate					Ecotoxicity			Human Health Effects				
	Phys ical Chem	Photo deg	Hydro lysis	Fuga city	Bio deg	Acute Fish Tox	Acute Invert Tox	Alga Tox	Acute Tox	Point Muta tions	Chrom Effects	Subchr onic	Repro/ Develop
26544-38-7	C	D/C	D	C	B	B	B	B	A	B	B	B	B
27859-58-1	C/T	D/C	D	C	A	T	T	A	B	T	T	T	T
25377-73-5	C	D/C	BD	C	B	B	B	B	A	B	B	B	B

- A Adequate data available
 B Bridging data from another category member
 C Computer modeling proposed
 D Technical discussion proposed
 T Test